

# BMS MIST strategy and experience

DV DMDG Rozman Symposium

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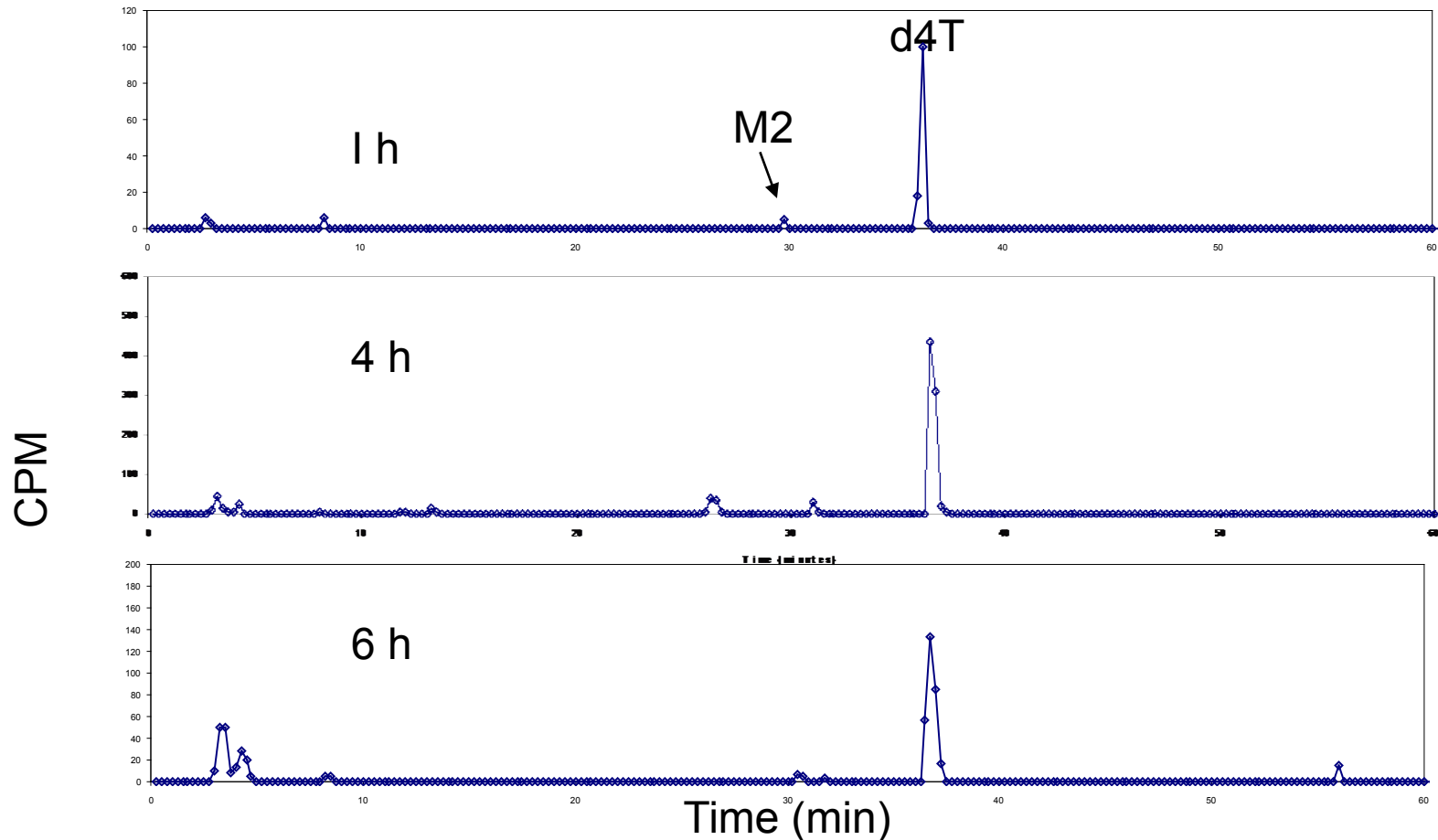
# Outline

- Intro
- BMS strategy to meet MIST
- Experiences and learnings
- How well has MIST met goals?

# Safety Testing of Drug Metabolites

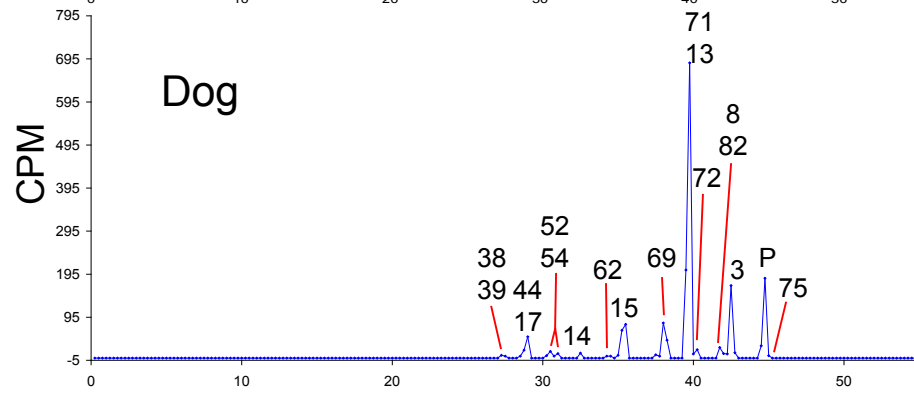
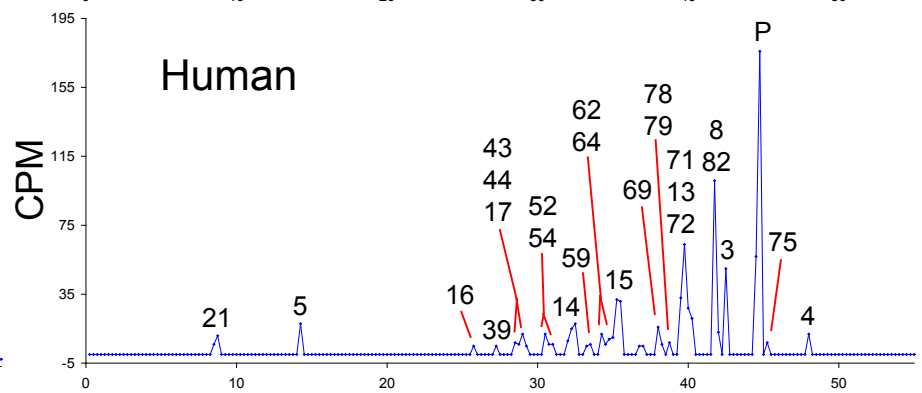
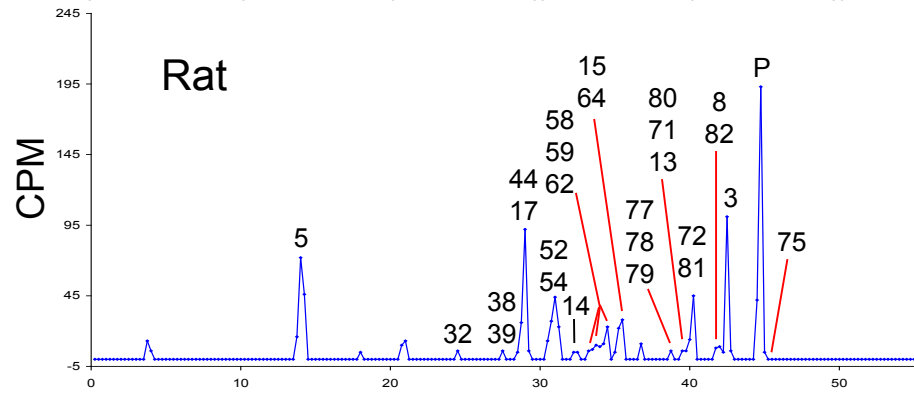
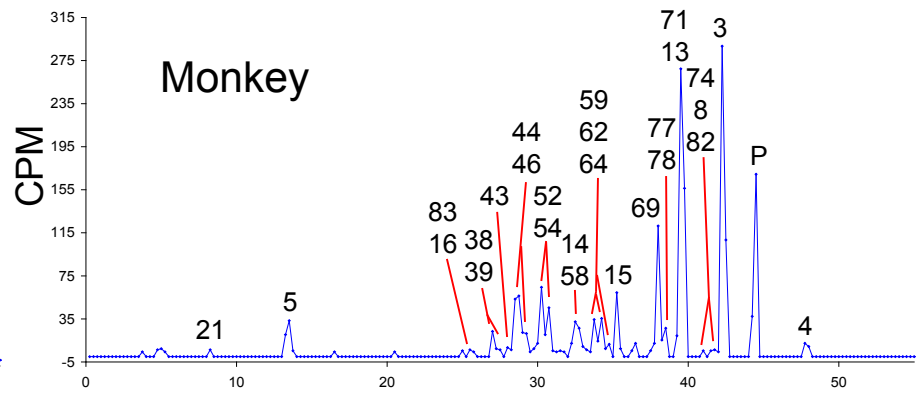
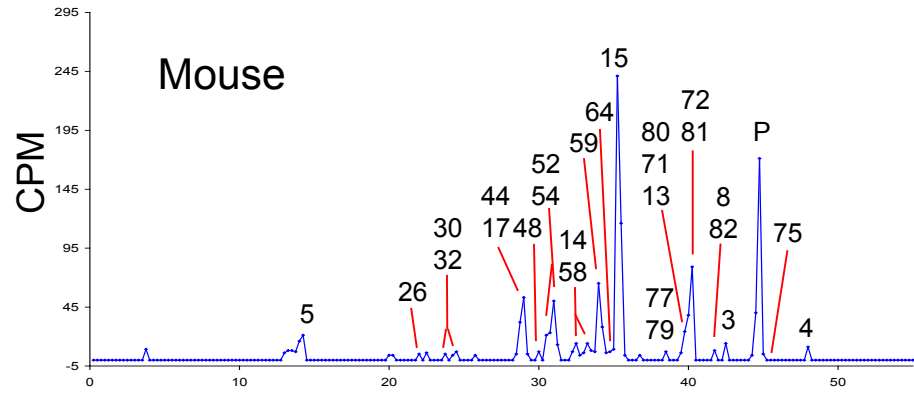
- Goal: Proper testing of the safety of a new drug requires that the metabolites that humans are exposed to are also tested in the species used for toxicological evaluation
  - Obligation to patient safety during clinical trials and post-approval
  - While the goal sounds simple in principle, in practice it can be rather complicated...need proper framework to set expectations

# Safety Testing of Drug Metabolites



• Sometimes the goal of ensuring adequate exposure to human metabolites can be fairly simple

# Safety Testing of Drug Metabolites



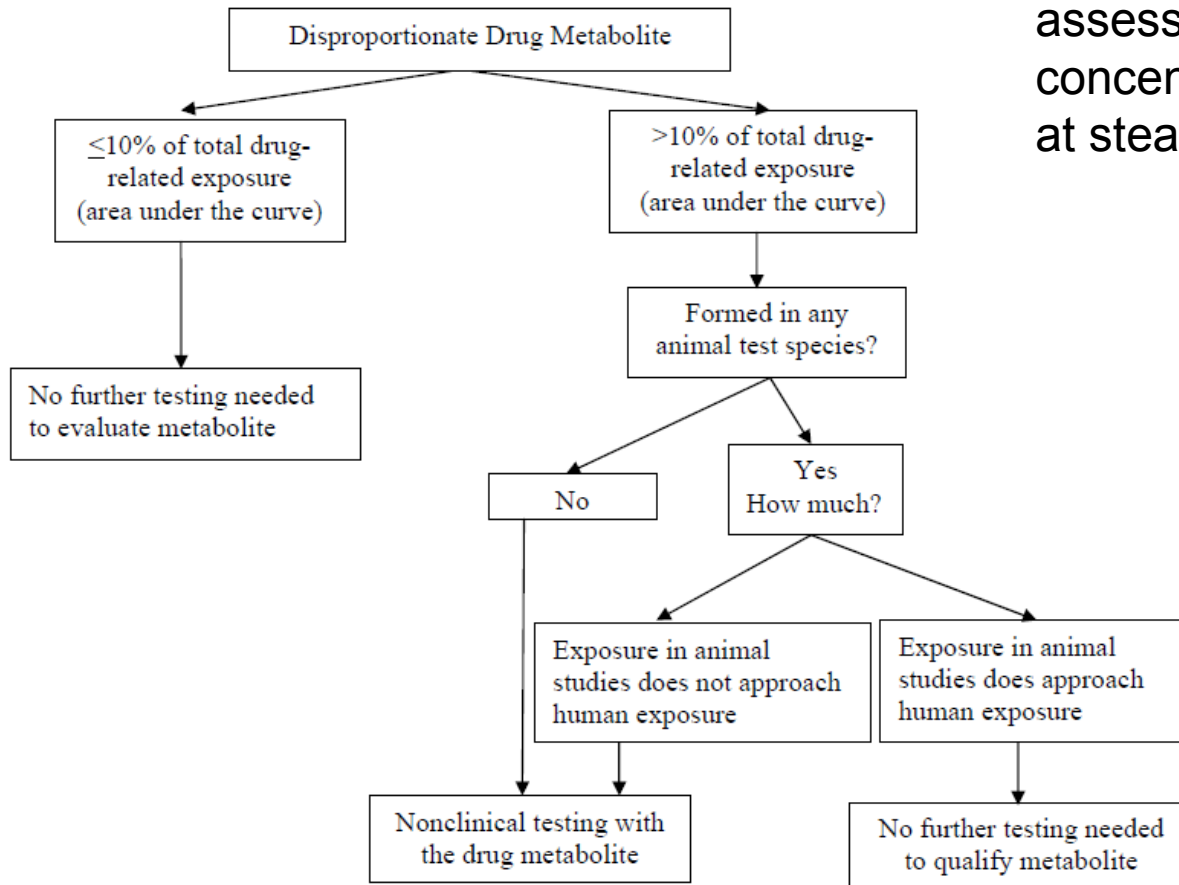
• Sometimes the goal of ensuring adequate exposure to the complex mixture of human metabolites can be very challenging!

# Safety Testing of Drug Metabolites

- History
  - Prior guidance from FDA
    - ICH S3A, March 1995
    - Carcinogenicity Study Protocol Submission, since 1997
  - PhRMA MIST task force, 1999-2002 (culminated in publication - Baillie et al, TAP, 182, 188-106, 2002)
  - PhRMA/FDA Joint workshop, Nov 2000
  - FDA Draft Guidance, June 2005
  - PhRMA/FDA/DruSafe Joint workshop, Nov 2005
  - **Safety Testing of Drug Metabolites Guidance for Industry Feb 2008**
  - **GUIDANCE ON NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS AND MARKETING AUTHORIZATION FOR PHARMACEUTICALS M3(R2), Dec 2009**
  - FDA revised guidance, Nov 2016

# FDA Safety Testing of Drug Metabolites Guidance for Industry

APPENDIX A:  
DECISION TREE FLOW DIAGRAM



“Generally, systemic exposure is assessed by measuring the concentration of the parent drug at steady state, in serum or plasma.”

# Other Considerations Relevant for Strategy Around Metabolite Characterization

- 1) On-target pharmacological activity of significant metabolites
- 2) Off target-related pharmacological activity of significant metabolites
- 3) Characterization of “other” off-target activity (including CYP and transporter liabilities) of major metabolites (DDI guidances)

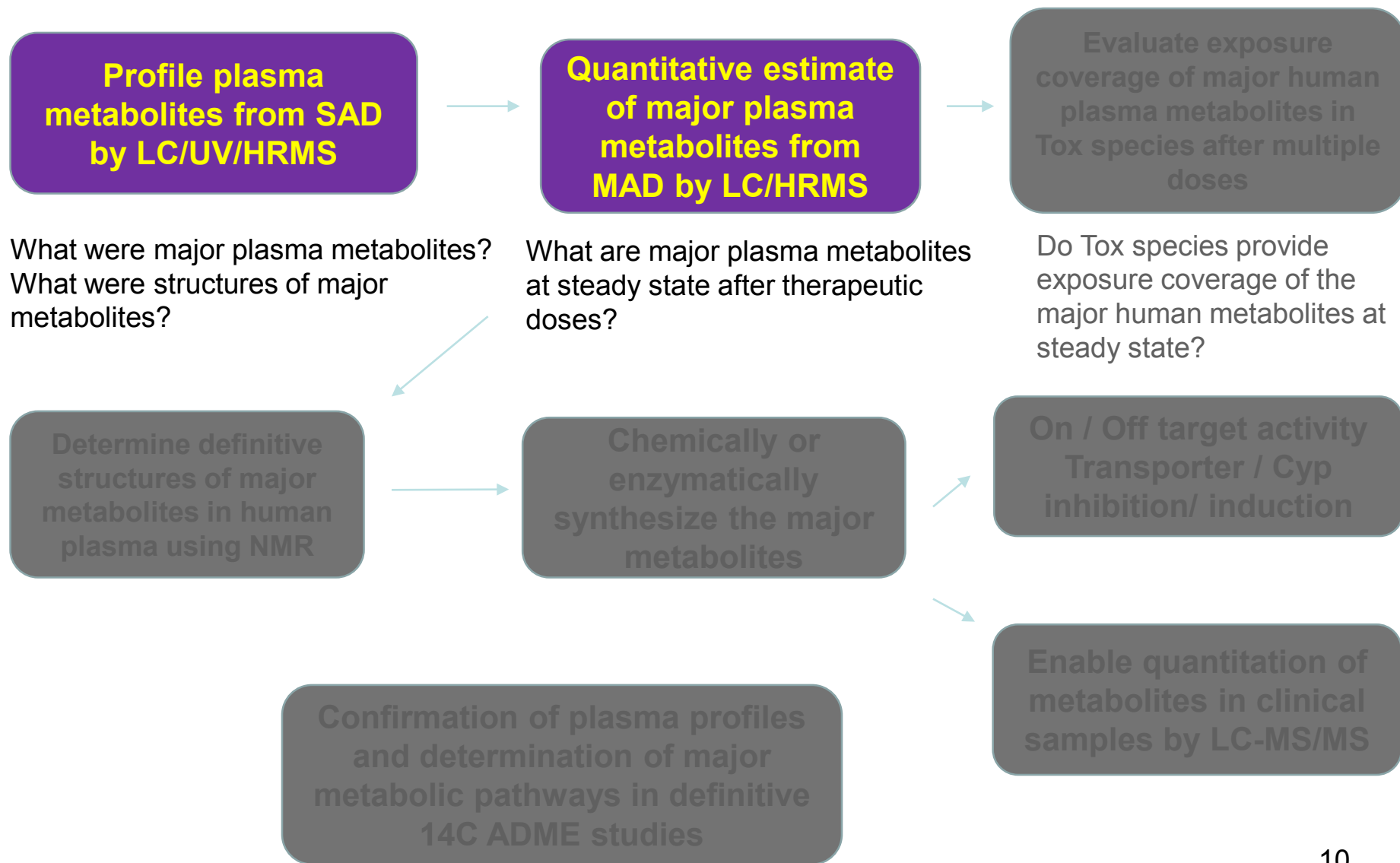


# Safety Testing of Drug Metabolites

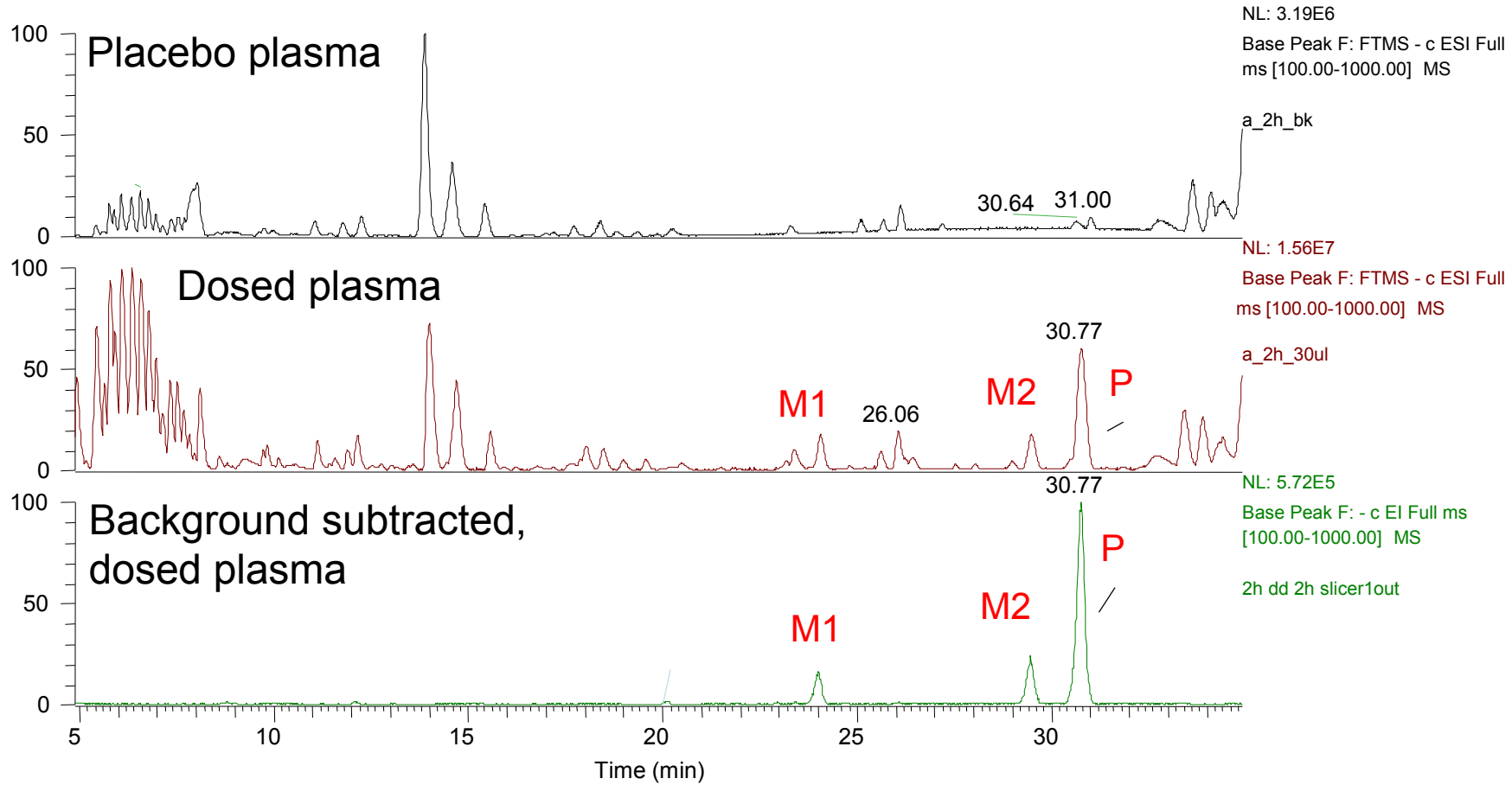
## BMS Strategy – Multi-tiered approach

- Determine metabolites with high probability to be circulating human metabolites from in vitro and animal in vivo studies
- Analyze metabolite profile present in samples from MAD study. Use HR-MS with data mining as front line tool to ensure robust metabolite detection
- Prepare samples from satellite animal groups, compare MS response of human metabolites to equivalent animal metabolite
- Employ multiple methods to estimate concentration of metabolites
- Characterize major metabolites:
  - Determine exposure in human and animals with fit for purpose analytical methodology
  - On and Off target activity as needed
- Final characterization in human ADME study, typically concurrent with phase 2

# General Approach for Characterizing Human Metabolites

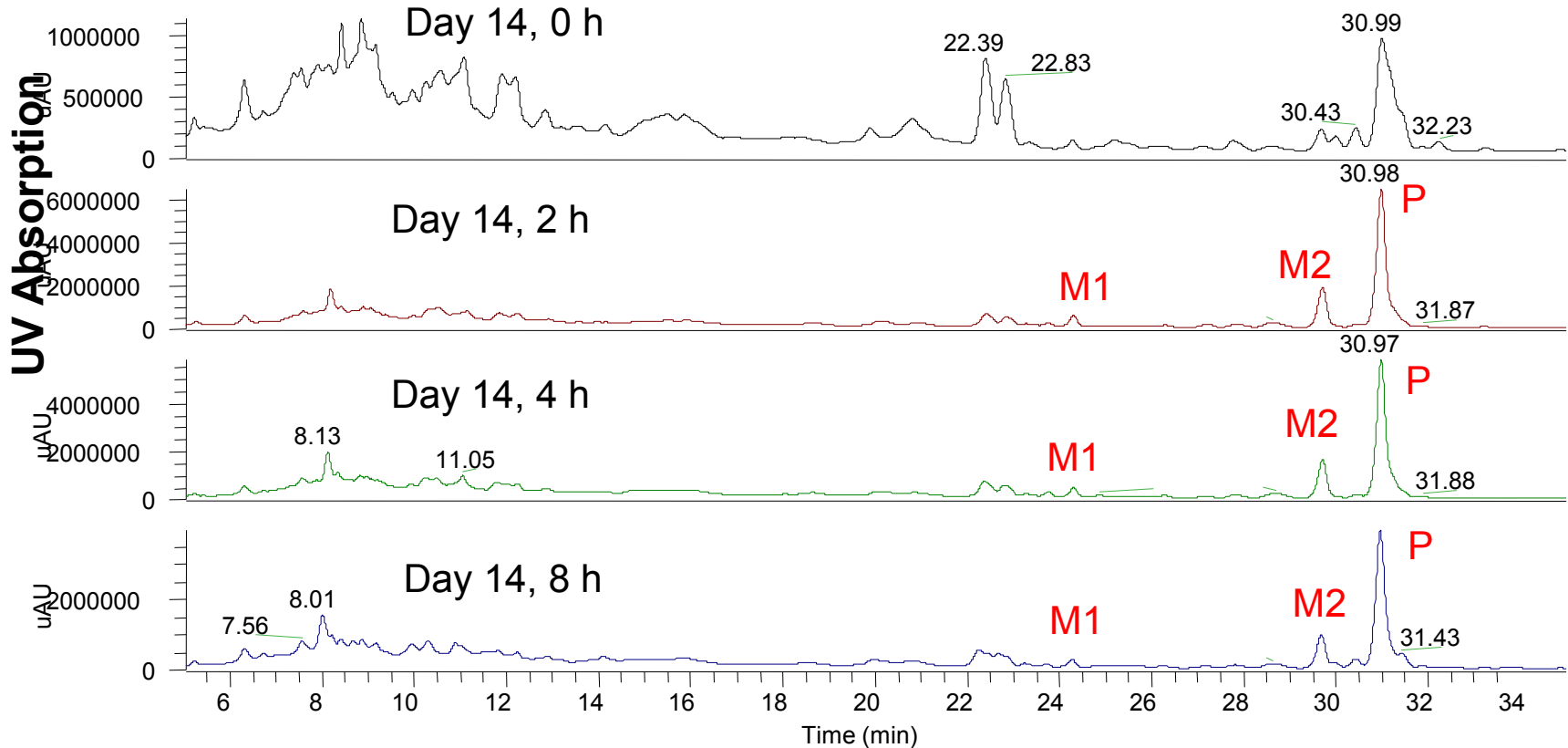


# Metabolite profiling in human plasma sample



- Background subtracted LC/HRMS profiles showed that M1 and M2 were significant plasma metabolites

# LC/UV profiles of human plasma (MAD)



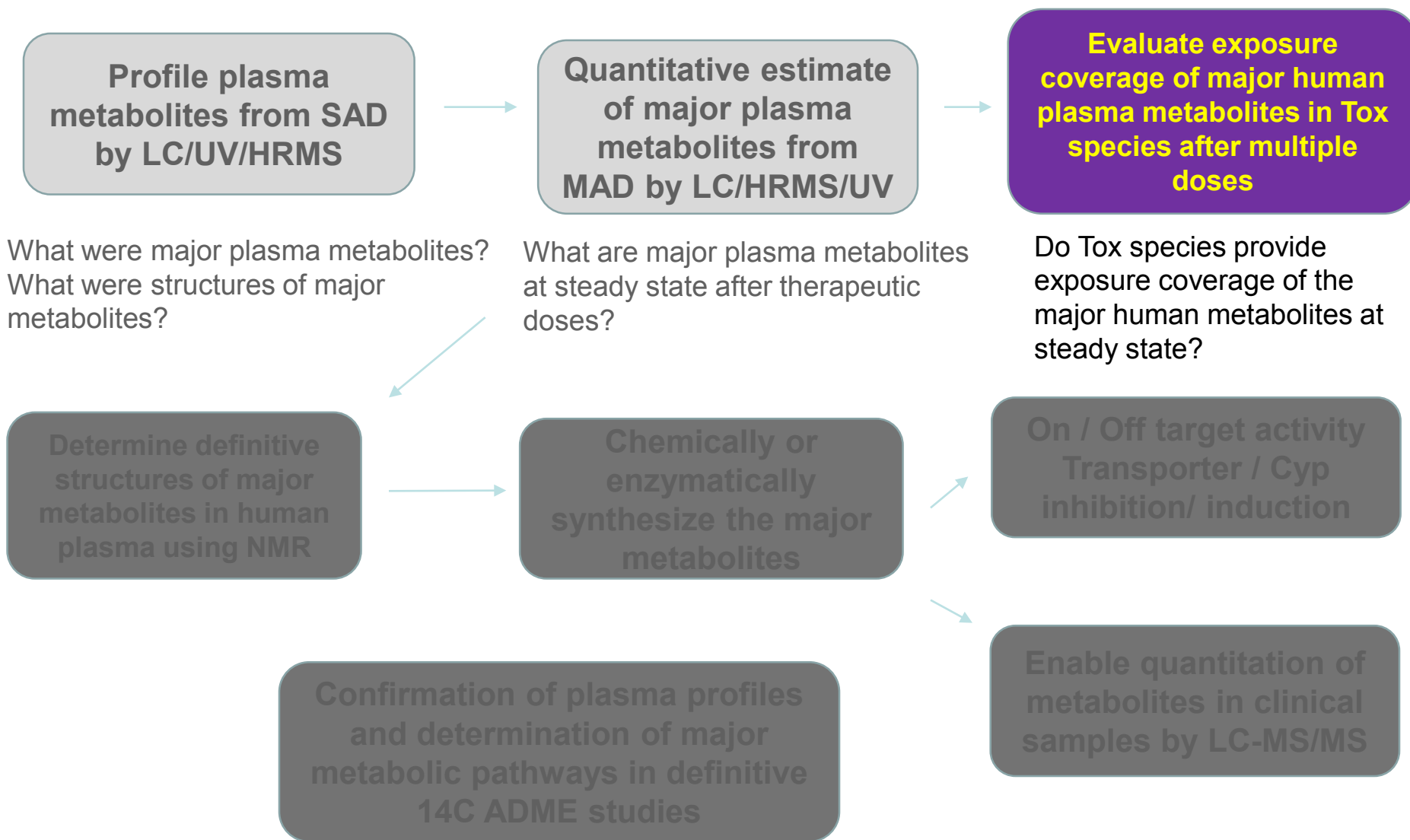
- **At steady state, M2 was the only metabolite that had an exposure greater than 10% of total drug related material in human**

# How to do initial quantitation from FIH samples?

## BMS Strategy for Quantitative Estimate – Use Multiple Approaches

- UV detection, especially if MS points to transformations expected to have a low impact on UV properties
- NMR quantitation of isolated sample, potential to use as a reference standard
- Employ radioactivity based quantitation from an in vitro or in vivo derived samples as a calibrant for MS response

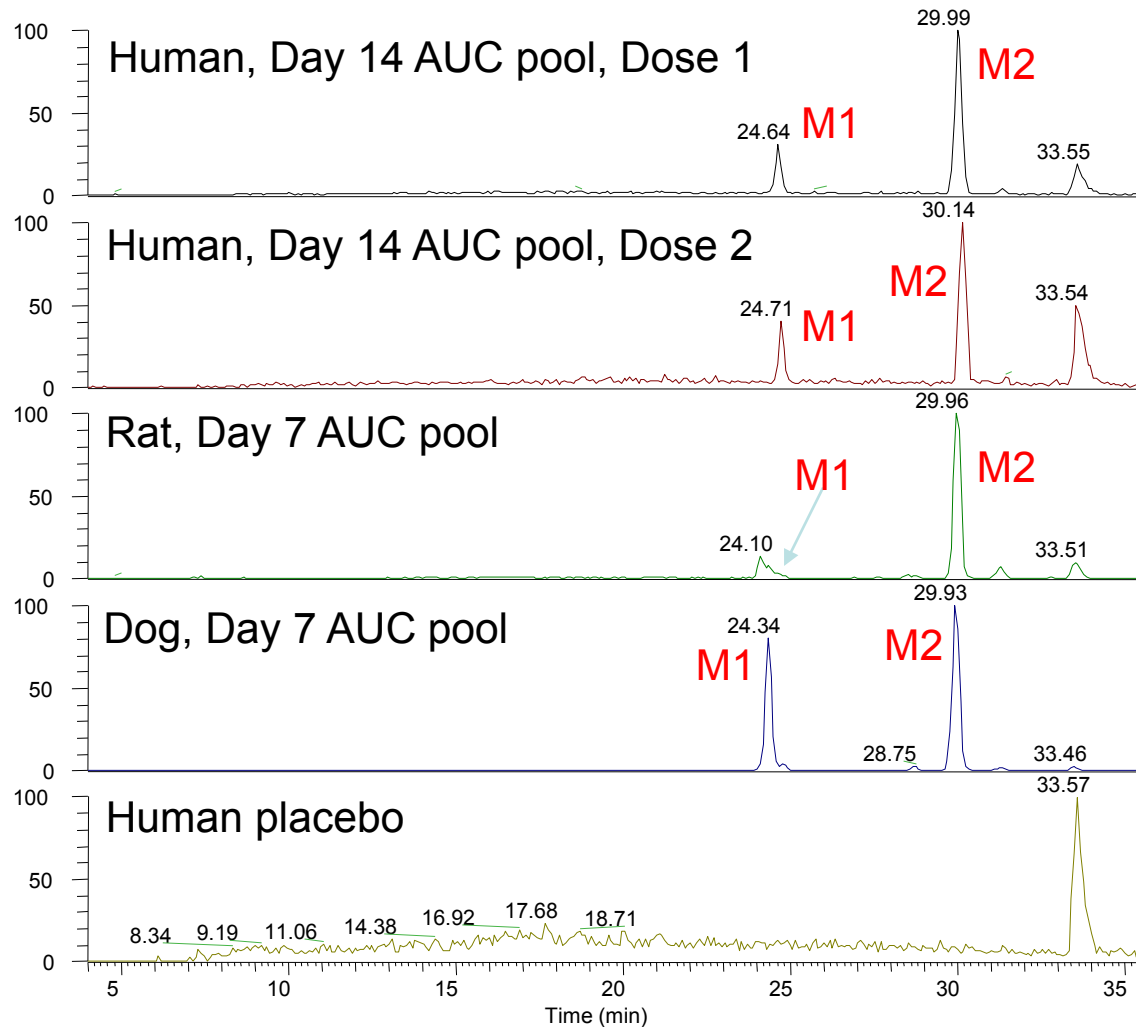
# General Approach for Characterizing Human Metabolites



# Comparison of Steady State Metabolite Exposures Without Use of Reference Standards

- Plasma samples from MAD study
- Plasma samples from steady multiple-day studies at NOAEL doses in relevant toxicology species
- For each species prepare one representative AUC-pooled sample
- Dilute the AUC-pooled sample from each species with blank plasma from the other 2 species (matrix match)
- Analyze peaks areas of metabolites by LC-HRMS
- Metabolite peak area ratio in animal vs human will represent the AUC ratio between the species

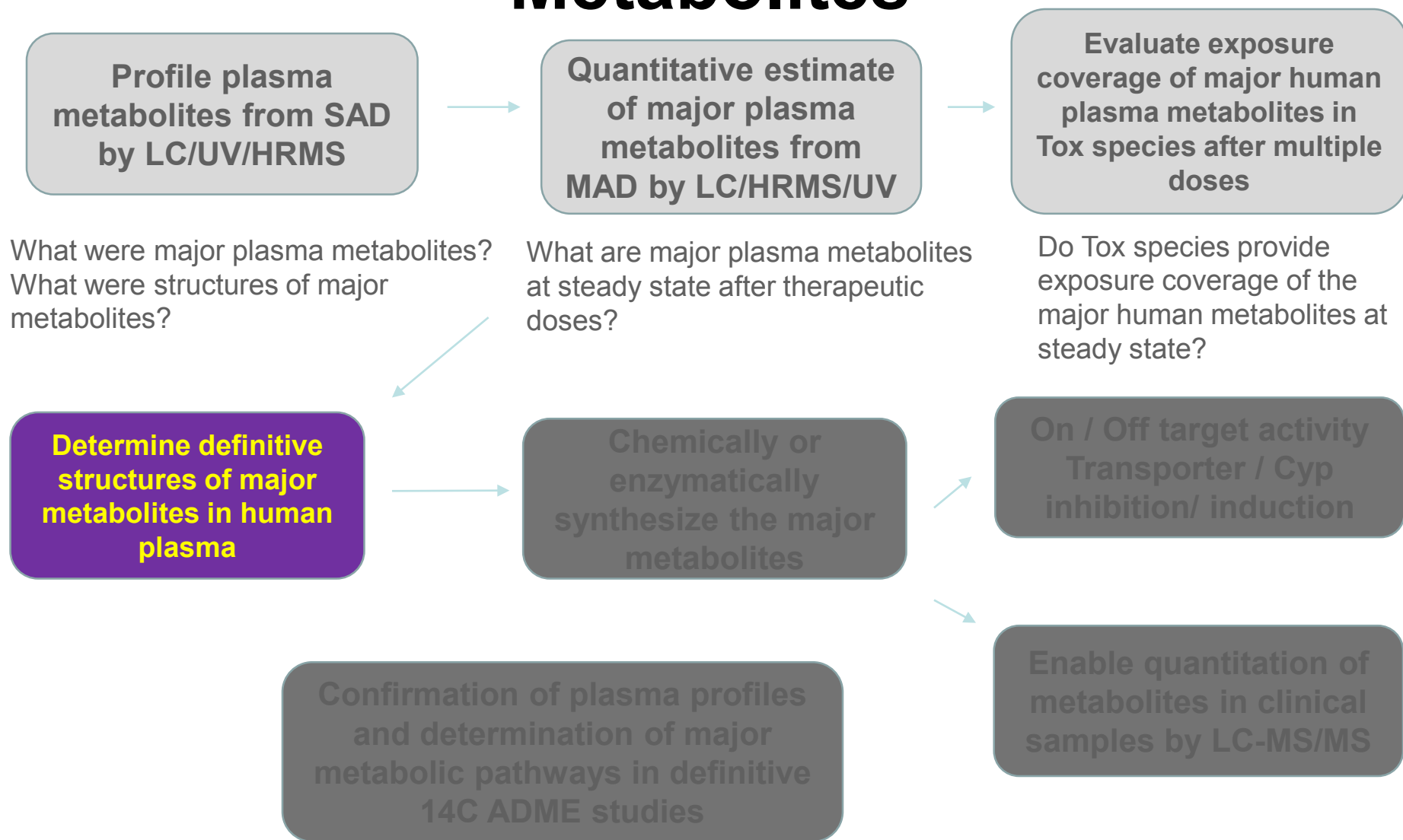
# Comparison of M1 & M2 exposures in AUC pooled samples from rat, dog and human



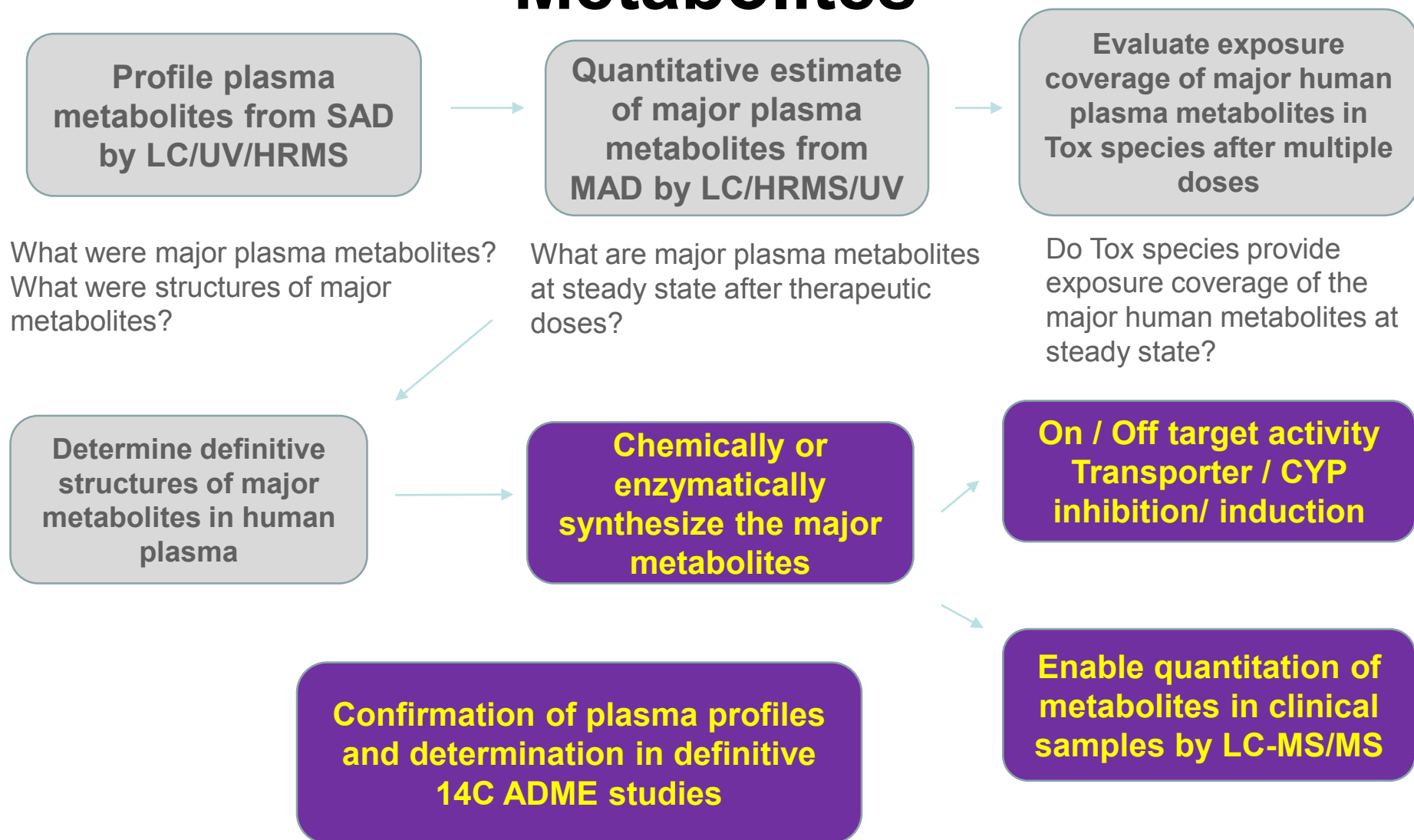
- **LC-HRMS, extracted ion chromatograms**
- **Peak ratios show coverage for M2 good in both species; coverage For M1 good in dog, challenging in rat**



# General Approach for Characterizing Human Metabolites



# General Approach for Characterizing Human Metabolites

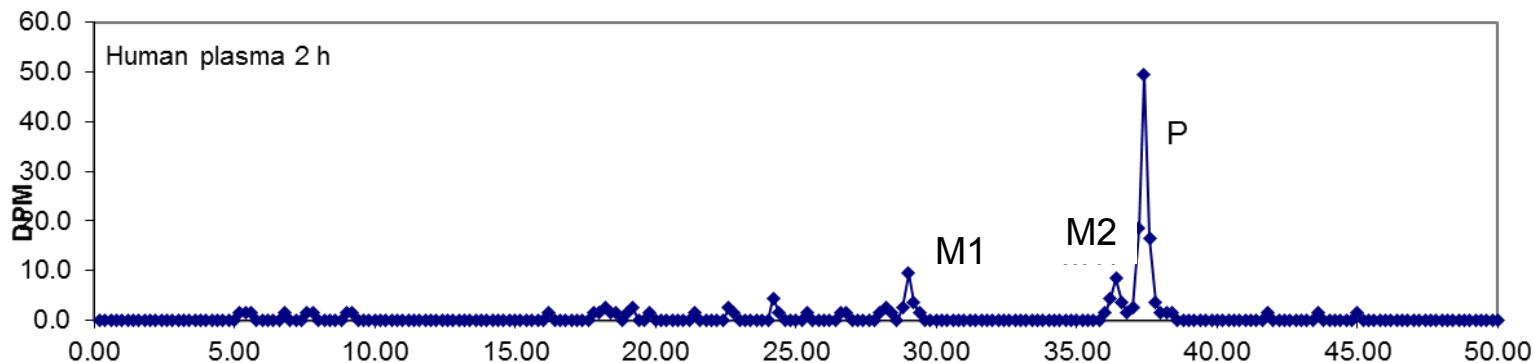


# Is it Worthy of GLP Bioanalysis?

## Tiered Approach Reduces Bioanalytical Workload

- At Crystal City May 2006 there was agreement that a tiered approach is possible for metabolite analysis
  - Not all assays must be GLP
  - Non-GLP LC/MS assays
- When seen as major metabolites in humans, GLP assays are usually developed, validated and used
  - Plasma exposures >10-25% threshold
  - Safety margin < 1
  - Profiling method may serve as the basis for GLP assay development

# Plasma Metabolite Profiles from <sup>14</sup>C-labeled Study in Human



- Radioprofiles confirm data from exploratory profiling showing M1, M2 were the only prominent metabolites in human after single dose

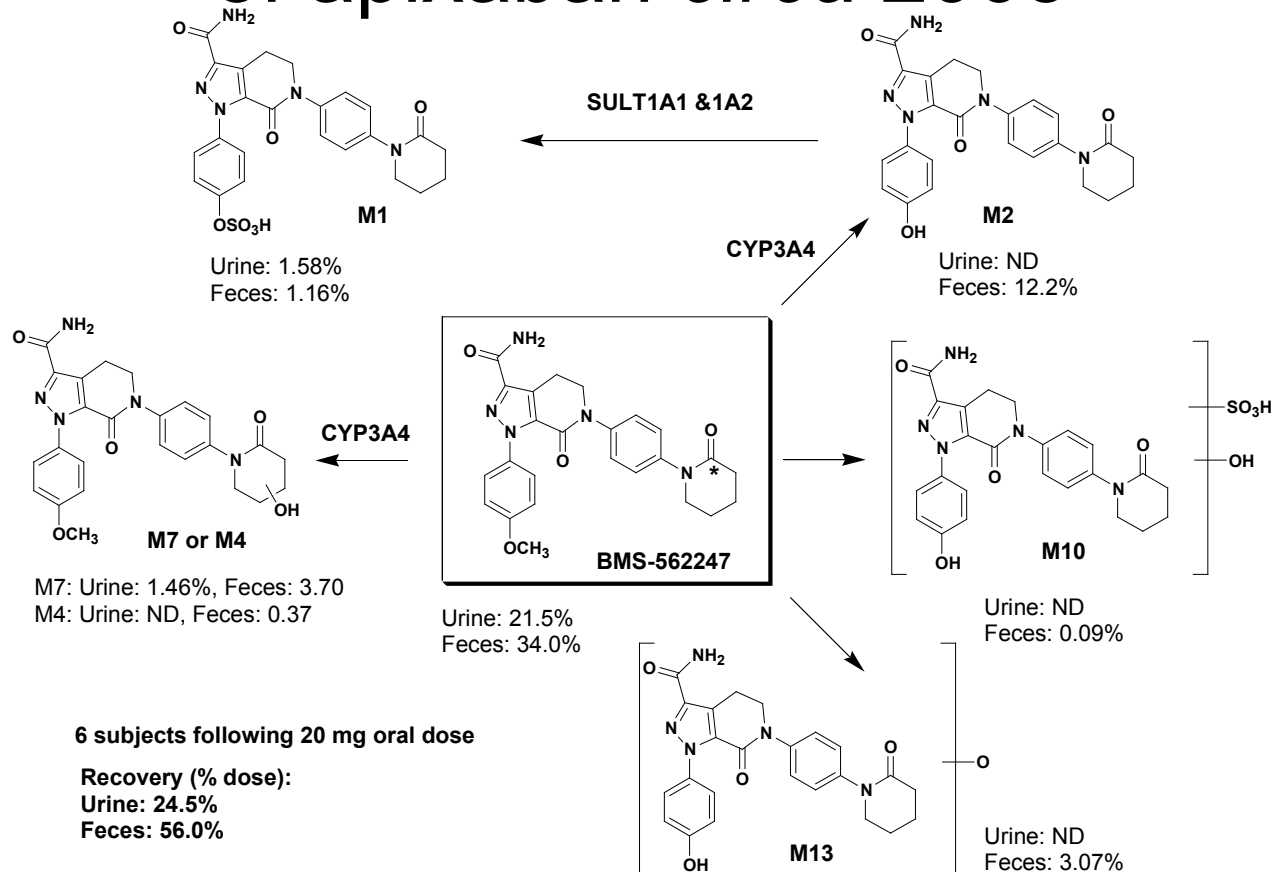
# Safety Testing of Drug Metabolites

- How has the strategy worked so far?
  - Allowed early determination of major circulating human metabolites and on average both reduced animals 14C-ADME studies and led to delay in 14C-human ADME to later in development
  - Only one instance of a late “surprise” in 14C-study in *ca.* 10 years of HRMS based screening
    - *Learning:* In case above, new metabolite would have been detected using negative ion electrospray. All FIH samples analysis now done on instrument with positive/negative switching

# Safety Testing of Drug Metabolites – coming up on 10 year anniversary!

- Has MIST (and ICH) lived up to its goals?
  - 1) Make drugs safer, both during clinical trials and post-marketing
  - 2) Give sponsors and regulatory agencies a common framework and set of expectations for evaluation of overall safety testing of a drug and its metabolites

# Pre-MIST experience...Metabolic pathways of apixaban *circa* 2005



- M1 accounts for approximately 20% of the circulating radioactivity in human subjects, 1-5% in animals

# Characterization of M1

Mean Cmax and AUC in toxicological species and humans at relevant doses

Species	Dose	Cmax (ng/mL)	AUC (ng/mL*h)
Rat (7 day)	600 mg/kg/day	45	197
Dog (7 day)	100 mg/kg/day	66	1204
Human (single dose)	20 mg (solution)	79	1829

- Initial HA interaction indicated a need to study M1 in a dedicated rat study



# Characterization of M1

- Potential for chemical reactivity
  - Sulfate conjugates with well characterized potential for chemical reactivity:
    - Conjugates of benzylic and allylic alcohols; aryl amines, hydroxylamines, and hydroxamic acids; secondary nitroalkanes
  - Phenolic sulfates, such as M1, are not known to be chemically reactive
    - No association of carcinogenicity/mutagenicity with phenolic sulfates
      - Drugs, endogenous substances and food constituents
    - Thermodynamically stable in aqueous solution
    - Chemical reactions limited to hydrolysis of conjugate to yield starting phenol
  - Quantum chemical calculations demonstrate that the energetics of formation of cationic reactive species from phenolic sulfates is highly unfavorable

# M1 Issue Resolution

- M1 was not a chemically reactive and did not inhibit factor Xa
- The enzymes predicted to be responsible for formation of M1 were not subject to significant polymorphic distribution
- C<sub>max</sub> exposure values of M1 in humans at clinical doses are similar to those found in rats and dogs at toxicological doses
- AUC exposure values of M1 in humans at clinical doses are similar to those found in dogs at toxicological doses
- Eventually the request to do direct animal studies with M1 was dropped and study not conducted... *Safety Testing of Drug Metabolites Guidance* likely would have led to a more rapid resolution of this request

# What biological activity will a metabolite likely possess?

- Typical metabolites are very closely related structurally to the parent, thus would be expected to have some degree of activity against the target receptor, i.e., it is not surprising that they would follow the same SAR as parent (*Humphreys and Unger, CRT, 19, 1564-1569*)
- Conversely, these closely related species would not necessarily be expected to gain new activities not seen with parent
  - Does this hypothesis hold true? Seems to be from the literature as there are few examples of where a stable metabolite produced toxicity through a pathway not impacted as well by the parent.

# What biological activity will a metabolite likely possess?

- How often do parent-metabolite pairs display significantly different off-target pharmacology?
  - Pairs such as terfenadine-fexofenadine are examples of parent-metabolite with significant off-target effects (HERG binding), however, the off-target activity resides with the parent
  - Metabolites can have significantly different physicochemical properties which may make them prone to physical deposition during elimination leading to toxicity (eg., guaifenesin induced urolithiasis)

# So why characterize stable metabolites?

- There is a reasonable chance they will contribute to the pharmacological activity, and may even drive a significant portion of the observed efficacy
- While examples of stable metabolites mediating off-target toxicity are relatively rare there are some notable recent examples:
  - An IQ working group confirmed that DDIs mediated by metabolites were rare (Yu H, et al. *DMD* (2015) 43:620-30), however, a Vertex clinical candidate demonstrated a very significant metabolite mediated CYP3A DDI (Zetterberg C, et al. *DMD* (2016) 44:1286-95)
  - A clinical candidate from Incyte was halted due to unexpected renal toxicity thought to be driven by the local crystallization of a human selective AO metabolite (Diamond S, et al. *DMD* (2010) 38:1277-85)

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